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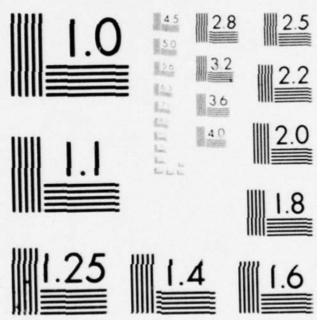

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Distribution of Tissue Water and Electrolytes in

Normal Rhesus Macaques

C. T. Liu, PhD, and M. J. Griffin

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Techniques for the determination of water and electrolytes in individual tissues of normal rhesus monkeys are described. Base-line values for intracellular and extracellular distribution of water and electrolytes in 14 different tissues including skin, skeletal muscle (gastrocnemius), cardiac muscle (left ventricle), lung, diaphragm, liver, renal cortex, outer medulla and inner medulla, cerebral cortex, cerebellum, thalamus-hypothalamus complex, medulla oblongata, and spinal cord are presented.

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SUMMARY

Techniques for the determination of water and electrolytes in individual tissues of normal rhesus monkeys are described. Base-line values for intracellular and extracellular distribution of water and electrolytes in 14 different tissues including skin, skeletal muscle (gastrocnemius), cardiac muscle (left ventricle), lung, diaphragm, liver, renal cortex, outer medulla and inner medulla, cerebral cortex, cerebellum, thalamus-hypothalamus complex, medulla oblongata, and spinal cord are presented.

The maintenance of body homeostasis is essential to life. Measurements of body fluid compartments,[10] renal functions,[9] blood pH, and distributions of water and electrolytes in body fluids and tissues are basic criteria for evaluating homeostatic conditions. Although the rhesus macaque has been often considered as an ideal model for medical research and some data are available about whole-body fluid compartment volumes,[10-12] little is known concerning the water and electrolyte content of individual tissues.[5,23] The purpose of this study was to establish base-line values for total, intracellular, and extracellular distributions of water and electrolytes in selected tissues of rhesus macaques.

Materials and Methods

Six healthy male rhesus macaques weighing 3-5 kg were adapted to chair restraint for 6 days prior to experimentation. During the period of adaption, monkey chow and water were provided ad libitum. These monkeys served as the uninfected normal controls during studies on the pathogenesis of rickettsial infection in monkeys.[12]

Experimentation was initiated by anesthetizing the rhesus macaque with Ketamine (40 mg/kg) and withdrawing a blood sample (10-15 ml) from the femoral artery. Thoracotomy was performed on the anesthetized monkey, and after interrupting the arterial circulation,[15,16] 14 different tissues of each monkey were taken within 10 minutes for determination of water content, electrolytes, and total lipids. The tissues included the hair-shaved skin, skeletal muscle (gastrocnemius), cardiac muscle (left ventricular), lung, diaphragm, liver, renal cortex, outer medulla, and inner medulla, cerebral cortex (predominantly grey matter), cerebellum, thalamus-hypothalamus complex, medulla oblongata, and spinal cord.

The blood was centrifuged and plasma was separated for determination of water content and electrolytes. Tissue water was determined by drying a small tissue sample (0.2-0.5 g) at 100 C for 48 hours to a constant weight by a method previously described.[15,16] Tissue electrolytes (Na^+ and K^+) were determined by flame photometry[15,20] after tissue homogenization in 10% trichloroactetic acid (1:10 wt/vol). Tissue Cl^- was extracted with distilled water and analyzed with a chloridometer.[3] Plasma electrolytes were determined by the same techniques as those for the tissue extracts. For the calculation of electrolyte concentration in terms of fat-free tissue, total lipids of each tissue were extracted

with 2:1 (vol/vol) chloroform-methanol.[4] For each gram of tissue, 20 ml of the mixed solvent was added for extraction. The tissue extract was further purified with distilled water and its lipid content was determined gravimetrically. Intra- and extracellular water and electrolyte distributions within each tissue were calculated according to equations reported by Benson et al.[1] (Table 1). A Donnan factor of 0.98 was used for the cardiac muscle, while a factor of 0.95 was utilized for all other tissues.[1] Further, tissue water and electrolyte values were expressed in terms of kilograms of fat-free wet tissue (FFWT) and fat-free dry tissue (FFDT).

Results

Data on the extra- and intracellular, and total water contained in 14 different tissues of the rhesus macaque are presented in Table 2. Total water ranged from 758 to 868 g/kg FFWT. In general, the nervous system, lung, and renal tissues contained more total water than other tissues. The highest total water was contained in the medulla oblongata, while the skin and diaphragm contained the lowest total water. Although intracellular water level was 2 to 3 times higher than extracellular water in most tissues, a reverse pattern was demonstrated in the lung, renal inner medulla and skin. Further, the thalamus-hypothalamus complex and renal inner medulla demonstrated the highest intra- and extracellular water, respectively.

Table 3 summarizes the distributions of intra- and extracellular electrolytes and total lipids in the various tissues. An increasing gradient of total and extracellular Na^+ from the renal cortex to the outer and inner medulla of the kidney was demonstrated. Among all tissues measured, the renal inner medulla yielded the highest values for total NaCl, extracellular Na^+ and K^+, and intracellular K^+. The medulla oblongata contained the highest total K^+. The skin showed the lowest value of total K^+ and the highest concentration of intracellular Na^+. The liver had the lowest value for intracellular Na^+. The skeletal muscle contained the lowest amount of total Na^+. The lowest concentrations of extracellular Na^+ and K^+, and total Cl^- were observed in the diaphragm. Further, the renal outer medulla contained the lowest amount of intracellular K^+.

Discussion

Extensive or comprehensive measurements of the distribution of water and electrolytes in individual tissues are rarely reported. The techniques employed are important for measuring intra- and extracellular changes during disease studies in monkeys and other animals. For example, alterations of tissue water and electrolytes have been studied in rhesus monkeys with Rocky Mountain spotted fever[12] and yellow fever.[11] Tissue water and electrolyte responses in dogs have been reported for hyperthermia,[26] deoxycorticosterone acetate administration,[13] hypocalcemic tetany,[16] respiratory acidosis and alkalosis,[27,28] and total-body x-irradiation.[15] Comparison has also been made of intracellular electrolytes between cardiac and skeletal muscle in rabbits.[21] Furthermore, effects of hemorrhagic shock and thyrotoxicosis on changes in the intra- and extracellular water and electrolyte concentrations of several tissues have been determined in baboons[29] and rats,[14] respectively.

Although tissue water and electrolyte data from normal rhesus monkeys have been obtained as incidental measurements by other investigators,[5,23] values of corresponding tissues are in good agreement with the present data. Other techniques for determining intracellular K^+, and tissue distribution of water and electrolytes include ion-exchange microelectrodes[6] and electron probe microanalysis.[7,17] However, the equipment for electron probe microanalysis is expensive and calibration procedures are difficult. In contrast, the application of Benson's techniques for determining tissue water and electrolytes, as described in this study, is simple and convenient. The basic principle for the calculations of tissue water and electrolytes distribution was based

on two assumptions: (1) the Cl^- space represents extracellular fluid volume[8,29] and (2) extracellular K^+ approximates intracellular Cl^-.[22]

The classic work of Skelton[24] reported total water content of several tissues in different species, including man. The present data on water content in corresponding tissues in the monkey are similar to the human data except that the water content in the brain and liver of the monkey is higher than in man. The determined differences between the skeletal muscle of diaphragm and gastrocnemius muscles, i.e., higher intracellular Na^+ and water and lower extracellular Na^+ and water in the diaphragm, may be explained by the continuing respiratory work performed by the diaphragm of monkeys.

As a rule, values for tissue intracellular water should be greater than those of extracellular water, and intracellular K^+ concentration must be higher than those of intracellular Na^+. The generalized cellular arrangement is seen in all measured tissues of rhesus monkeys except for the skin, lung, and renal medulla; this discrepancy may be caused by their specific cellular functions and structures. For instance, renal medulla is the site of a gradual increase in Na^+ gradient for urine concentration under the influence of antidiuretic hormone.[2,30] This unique phenomenon was demonstrated in the monkey's kidney by our techniques. The lung is composed of many alveoli, lined only with thin layers of endothelial and epithelial cells.[25] The main function for transport of gases (CO_2 and O_2) across the cell membranes may alter the membrane functions for maintaining cellular adsorption of K^+ and rejection of Na^+. The compact cellular layers of skin consist of epidermis and dermis with sweat glands. The unusually high content of cellular Na^+ and low K^+ in the monkey skin was seen in the skin of dogs.[13,18]

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TABLE 1--Equations for Calculating Intracellular and Extracellular Water and Electrolytes (using K as an example)

Variable	Equation
Extracellular water	$(H_2O)_E = 1000 \cdot (Cl)_T - [K]_E \cdot (H_2O)_T/[Cl]_E - [K]_E$
Intracellular water	$(H_2O)_I = (H_2O)_T - (H_2O)_E$
Extracellular K	$[K]_E = (K)_P \cdot$ Donnan factor
	$(K)_E = [K]_E \cdot (H_2O)_E/1000$
Intracellular K	$(K)_I = (K)_T - (K)_E$
	$[K]_I = 1000 \cdot (K)_I/(H_2O)_I$

E = *extracellular*; T = *total*; I = intracellular; P = plasma.

$(K)_P$ = plasma concentration of K (mEq/liter).

$[K]_E$ = extracellular K (mEq)/kg of extracellular water.

$(K)_E$ = extracellular K (mEq)/kg of fat-free wet tissue.

$(K)_I$ = intracellular K (mEq)/kg of fat-free wet tissue.

$[K]_I$ = intracellular K (mEq)/kg of intracellular water.

TABLE 2--Water Content in Various Tissues of Normal Rhesus Monkeys*

Tissue	H_2O Content (g/kg Fat-Free Wet Tissue)		
	Total	Extracellular	Intracellular
Cerebral cortex	859 ± 4	280 ± 37	579 ± 35
Cerebellum	835 ± 10	175 ± 25	660 ± 32
Thalamus-hypothalamus	857 ± 14	154 ± 44	703 ± 35
Medulla oblongata	868 ± 11	230 ± 23	638 ± 19
Spinal cord	831 ± 23	184 ± 40	647 ± 61
Heart	794 ± 15	197 ± 25	597 ± 26
Lung	826 ± 6	455 ± 49	371 ± 52
Liver	772 ± 12	238 ± 11	534 ± 12
Renal cortex	827 ± 7	305 ± 48	522 ± 50
Renal outer medulla	823 ± 3	153 ± 17	670 ± 18
Renal inner medulla	836 ± 8	599 ± 29	237 ± 24
Diaphragm	764 ± 9	108 ± 16	656 ± 15
Skeletal muscle	791 ± 10	161 ± 13	630 ± 11
Skin	758 ± 12	506 ± 79	252 ± 87

*Values are means ± SE.

TABLE 3--Na^+, K^+, Cl^-, and Total Lipid Concentrations in Various Tissues of Rhesus Macaques*

Tissue	$(Na)_T$ mEq/kg FFWT	$(Na)_T$ mEq/kg FFDT	$(Na)_E$ mEq/kg FFWT	$[Na]_I$ mEq/kg H_2O	$(K)_T$ mEq/kg FFWT	$(K)_T$ mEq/kg FFDT	$(K)_E$ mEq/kg FFWT	$[K]_I$ mEq/kg H_2O	$(Cl)_T$ mEq/kg FFWT
Cerebral cortex	57.8 ± 3.2	411 ± 24	38.2 ± 5.1	29.7 ± 5.9	94.8 ± 11	678 ± 85	1.02 ± 0.14	161 ± 19	37.0 ± 4.6
Cerebellum	51.9 ± 2.6	310 ± 25	24.9 ± 3.2	37.7 ± 6.6	95.1 ± 2.1	577 ± 28	0.65 ± 0.10	143 ± 9	25.7 ± 2.8
Thalamus-hypothalamus	59.2 ± 1.7	438 ± 50	21.6 ± 5.9	52.6 ± 5.5	94.3 ± 2.9	691 ± 66	0.54 ± 0.14	135 ± 8	21.3 ± 5.0
Medulla oblongata	54.5 ± 2.0	423 ± 27	32.6 ± 2.9	33.5 ± 6.7	103 ± 4.0	803 ± 54	0.83 ± 0.08	162 ± 9	30.8 ± 2.6
Spinal cord	79.3 ± 3.1	462 ± 51	25.9 ± 5.6	82.1 ± 4.8	82.3 ± 6.1	516 ± 75	0.70 ± 0.17	132 ± 19	23.6 ± 5.4
Heart	43.5 ± 3.1	218 ± 22	29.1 ± 3.5	24.1 ± 2.5	71.4 ± 6.2	351 ± 28	0.74 ± 0.11	120 ± 13	26.7 ± 3.2
Lung	87.1 ± 2.2	505 ± 15	65.1 ± 7.4	50.5 ± 14	52.3 ± 0.8	304 ± 14	1.64 ± 0.18	151 ± 22	57.6 ± 5.5
Liver	42.2 ± 2.1	186 ± 9	30.2 ± 3.8	21.7 ± 5.8	78.2 ± 1.9	349 ± 23	0.86 ± 0.07	144 ± 6	31.5 ± 1.6
Renal cortex	58.4 ± 1.3	340 ± 15	34.8 ± 7.1	40.6 ± 9.0	65.2 ± 1.6	378 ± 10	1.22 ± 0.21	128 ± 10	39.6 ± 6.0
Renal outer medulla	70.1 ± 7.5	394 ± 38	23.6 ± 0.9	71.5 ± 11.5	63.0 ± 3.8	355 ± 22	0.59 ± 0.04	95 ± 6	21.2 ± 1.9
Renal inner medulla	95.6 ± 5.2	585 ± 25	75.7 ± 11.6	76.8 ± 5.7	59.4 ± 2.5	367 ± 28	1.94 ± 0.27	231 ± 48	74.9 ± 2.4
Diaphragm	49.1 ± 4.4	208 ± 16	12.9 ± 2.6	53.6 ± 7.5	69.6 ± 4.1	298 ± 24	0.50 ± 0.16	132 ± 9	15.5 ± 2.0
Skeletal muscle	39.2 ± 2.6	190 ± 16	20.3 ± 2.8	32.1 ± 8.2	84.5 ± 4.6	405 ± 16	0.53 ± 0.09	132 ± 9	22.2 ± 1.7
Skin	104 ± 10	431 ± 40	68.3 ± 13	102 ± 7	24.7 ± 1.2	105 ± 8	1.49 ± 0.35	105 ± 42	64.3 ± 10.2

*Values are means ± SE (n = 6); n = 5; Plasma concentration (mEq/liter); Na = 141.3 ± 5.7, K = 3.41 ± 0.25, Cl = 110 ± 1.3.
FFWT = Fat-free wet tissue. FFDT = Fat-free dry tissue. WT = Wet tissue.

, and Total Lipid Concentrations in Various Tissues of Rhesus Macaques*

a)$_T$ /kg WT	$(Na)_T$ mEq/kg FFDT	$(Na)_E$ mEq/kg FFWT	$[Na]_I$ mEq/kg H_2O	$(K)_T$ mEq/kg FFWT	$(K)_T$ mEq/kg FFDT	$(K)_E$ mEq/kg FFWT	$[K]_I$ mEq/kg H_2O	$(Cl)_T$ mEq/kg FFWT	$(Cl)_T$ mEq/kg FFDT	Total Lipid g/kg WT
± 3.2	411 ± 24	38.2 ± 5.1	29.7 ± 5.9	94.8 ± 11	678 ± 85	1.02 ± 0.14	161 ± 19	37.0 ± 4.6	226 ± 36	72.9 ± 7.4
± 2.6	310 ± 25	24.9 ± 3.2	37.7 ± 6.6	95.1 ± 2.1	577 ± 28	0.65 ± 0.10	143 ± 9	25.7 ± 2.8	147 ± 17	63.8 ± 13.6
± 1.7	438 ± 50	21.6 ± 5.9	52.6 ± 5.5	94.3 ± 2.9	691 ± 66	0.54 ± 0.14	135 ± 8	21.3 ± 5.0	172 ± 52	144 ± 6
± 2.0	423 ± 27	32.6 ± 2.9	33.5 ± 6.7	103 ± 4.0	803 ± 54	0.83 ± 0.08	162 ± 9	30.8 ± 2.6	245 ± 33	126 ± 18
± 3.1	462 ± 51	25.9 ± 5.6	82.1 ± 4.8	82.3 ± 6.1	516 ± 75	0.70 ± 0.17	132 ± 19	23.6 ± 5.4	124 ± 18	130 ± 20
± 3.1	218 ± 22	29.1 ± 3.5	24.1 ± 2.5	71.4 ± 6.2	351 ± 28	0.74 ± 0.11	120 ± 13	26.7 ± 3.2	132 ± 20	25.0 ± 1.2
± 2.2	505 ± 15	65.1 ± 7.4	50.5 ± 14	52.3 ± 0.8	304 ± 14	1.64 ± 0.18	151 ± 22	57.6 ± 5.5	333 ± 31	23.8 ± 3.8
± 2.1	186 ± 9	30.2 ± 3.8	21.7 ± 5.8	78.2 ± 1.9	349 ± 23	0.86 ± 0.07	144 ± 6	31.5 ± 1.6	142 ± 11	39.5 ± 4.9
± 1.3	340 ± 15	34.8 ± 7.1	40.6 ± 9.0	65.2 ± 1.6	378 ± 10	1.22 ± 0.21	128 ± 10	39.6 ± 6.0	229 ± 34	24.1 ± 2.9
± 7.5	394 ± 38	23.6 ± 0.9	71.5 ± 11.5	63.0 ± 3.8	355 ± 22	0.59 ± 0.04	95 ± 6	21.2 ± 1.9	120 ± 11	28.9 ± 3.0
± 5.2	585 ± 25	75.7 ± 11.6	76.8 ± 5.7	59.4 ± 2.5	367 ± 28	1.94 ± 0.27	231 ± 48	74.9 ± 2.4	465 ± 41	41.3 ± 5.3
± 4.4	208 ± 16	12.9 ± 2.6	53.6 ± 7.5	69.6 ± 4.1	298 ± 24	0.50 ± 0.16	132 ± 9	15.5 ± 2.0	61 ± 9	21.6 ± 2.4
± 2.6	190 ± 16	20.3 ± 2.8	32.1 ± 8.2	84.5 ± 4.6	405 ± 16	0.53 ± 0.09	132 ± 9	22.2 ± 1.7	108 ± 11	12.6 ± 2.0
± 10	431 ± 40	68.3 ± 13	102 ± 7	24.7 ± 1.2	105 ± 8	1.49 ± 0.35	105 ± 42	64.3 ± 10.2	279 ± 52	63.0 ± 14.8

SE (n = 6); n = 5; Plasma concentration (mEq/liter); Na = 141.3 ± 5.7, K = 3.41 ± 0.25, Cl = 110 ± 1.3.
tissue. FFDT = Fat-free dry tissue. WT = Wet tissue.